

Claims

1. Oligonucleotide for genotyping and pathotyping the species *Pseudomonas aeruginosa* with a nucleic acid sequence, selected from the group consisting of (all sequences in 5' → 3' direction):

i)

GAAGCCCAGCAATTGCGTGTTTC
GAAGCCCAGCAACTGCGTGTTTC
GGTGCTGCAGGGTGTTTCGCCGG
GGTGCTGCAGGGCGTTTCGCCGG
CAAGATCGCCGCAGCGGTCAAC
CAAGATCGCCGCTGCGGTCAAC
TGCTGCTGGCGGCGGTGTGCTAT
TGCTGCTGGCAGCGGTGTGCTAT
CCTCGCCCTGTTCCCGCCGCTCTGG
CTCGCCCTGTTCCCGCCGCTCTGG
TCGAGCAACTGGCAGAGAAATCCG
CGAGCAACTGGCGGAGAAATCCG
GCGGAAAACCTTCCTGCACATGATGTT
GCGGAAAACCTTCCTCCACATGATGTT
AGCTCAGCAGACTGCTGACGAGG
AGCTCAGCAGACCGCTGACGAG
AAGAGGACGGCCGCCGGGTGACGCC
AAGAGGACGGCCGCCAGGTGACGCCG
GACAAGATGCGCCTCGACGACC
GACAAGATGCGTCTCGACGACCG
AGCCGACCTACGCGCCGGGCAG
CAGCCGACCTATGCGCCGGGCAG
CCGTTCGAACGGCTCATGGAGCA
GCCGTTCGAACGACTCATGGAGCA
TGGAGCAGCAAGTGTTCCCGGC
TGGAGCAGCAACTGTTCCCGGC

GAACAAGACCGGTTCCACCAACGG
AACAAGACCGGCTCCACCAACGG
GCGACCTGGGCCTGGTGATCCT
GCGACCTGGGACTGGTGATCCT
GCCGACCAACTGAACTCCAACCTCG
GTCGCTGAACGGCACCTACTTCA
CAGCCTGCGGTCATGTCCTCGG
CGCCAGTTTGAGAACGGAGTCACC
GCGCGATCTTCTCCACTTCATCGG
GCCTCCGCGATTGAACATCGTGAT
GTAGCCGGAGTCGAGCGGAATCAT
GTGAGCATGGAATCGGCAGTCGTT
CGAGGAGTTTCGGACCCGCTTTGA
AATAGGACCGGCAGAACGGGCATT
GCGCCTTCTCCTCTTTGCAGATGT
CAGTATGGTACGGACACGAAGCGC
GCATCATTGCGCGTCACATCTGGT
TCTGAACTGCGGCTATCACCTGGA
AATTGATGGCTTCTCAGGCGCAGG
AGTCATGGGACTGAATACGGCGACT
TTCTCGGTGTCGAGGGATTCTCGG
TGGTAGCTCTCGACGTACTGGCTG
CCCGTTGCTCATAACCCGTTCTG
AGGGCATTCTCAGGTGGACTCAGG
ACCTGTGTCGCTGGAGGGTATGTT
AGCGTCCCTGACCAACCTCATCAG
CGCCAACAATTCGCCATTACAGCG
TCCAACAGGCAGGAGTACAGGGTG
CGCTGCACATACAGGTCCGTTCTC
AGCCCAGCAATTGCGTGTTTCTCCG
AGCCCAGCAACTGCGTGTTTCTCC
GCTGCTGGCGGCGGTGTGC
TGCTGCTGGCAGCGGTGTGCT

CAGAAAGCTCAGCAGACTGCTGACGAG
GAAAGCTCAGCAGACCGCTGACGAG
ACGGCCGCCGGGTGACGCC
ACGGCCGCCAGGTGACGCCG
GCCGACCTACGCGCCGGGC
AGCCGACCTATGCGCCGGGCA
GTTCGAACGGCTCATGGAGCAGCA
GTTCGAACGACTCATGGAGCAGCAAG
CAGCCCAGTCAGGACGCGCA
AGTGACGTGCGTTTCAGCAGTCCC
GTGTCACGGCCCATGTCTAGCAGC
CGAAGTCTGAGGTGTGGACCCGC
CGCTGGAGGGTATGTTCCGCAAGG
CGTACTCAGCTTCTCCACCCAGCG
CCTGGACCTCTCCAAGGTTCGCCT
GCCATTCCGACGACCAAACAAGGC
GTGCTGCAGGGTGTTTCGCCG
GCTGCAGGGCGTTTCGCCG
CAAGATCGCCGCAGCGGTCAACGAC
CAAGATCGCCGCTGCGGTCAACGAC
GCTCAGCAGACTGCTGACGAGGCTAACG
GCTCAGCAGACCGCTGACGAGGCTAAC
CGACCTACGCGCCGGGCAG
CGACCTATGCGCCGGGCAGC
CGTTCGAACGGCTCATGGAGCAG
CGTTCGAACGACTCATGGAGCAGC
CGACCTGGGCCTGGTGATCCT
GCGACCTGGGACTGGTGATCCTGG
CAGTTGTCGCCAGGTCTGGAGAATCC
CACATCAATGTCAGCCCACGCCA
CTGGAGCCTGCGAAAGTGGCTC
ACGAGGGTGATGGCTGGGAATACG
GCCAATTGGGTCAGCAAGCAACG

CGTGTCGCGAACTCGCATGGC
AGGCCATGGGCTAGCCGGATGC
CGAAGCGTAGGGTCTTCGTAGCC
TGCGAGGACCAGAAACCTTGATGG
CGGTATGAAGATGGGTGGTTGGGTCG
CCTGAATCCGACCATTCGCGAGTC
TCGGACTGTACTCCTACGAAGCAGC
CCAATCCCTATCGCTGGAACCGTACC
GCTCGGGACTCGCATTTTCGTCC
GCGTTATTGCTCGGTCTCTCCTCG
TGCATAGGAGTCATGCCGACAGCA
GCCTGCCTACTTGTTCCCAACGC
GGCTGTATTGCCCCGCCATTCTCC
CGACAGACAGAAAGGGTTCTTGCGC
CACCATGCAAATGCTCGATGGACTGC
GCAGGCGTCCAAGTTGGAGCTCTCC
GGAACACAACGTGGGGCGTGAC
CCAGTTGGCACCACCATGCTTGC
GACCGCAAGCAGAAACGGCATGC
CCATGGTCGGAACAGGCACGATATGC
CCACTCGATCATGTTGAGCATCGGCTCC
GGTTAGTCCCTTCTGCCCCGCATCG

- ii) oligonucleotides matching one of the oligonucleotides under i) in at least 60%, preferably in at least 80%, and particularly preferably in at least 90%, 92%, 94 %, 96% of the bases and allowing specific hybridization with nucleic acid sequences of bacterial strains of the species *Pseudomonas aeruginosa*,
- iii) oligonucleotides differing from one of the oligonucleotides under i) and ii) in that they are extended by at least one nucleotide, and
- iv) oligonucleotides hybridizing with a sequence, which is complementary to an oligonucleotide under i), ii) and iii), under stringent conditions.

2. Microarray device comprising a support element, on which oligonucleotide probes are immobilized on predetermined regions, for specifically detecting bacterial strains of the species *Pseudomonas aeruginosa*.

3. Device according to claim 2, characterized in that the device is a reaction tube having a shape and / or size typical for a laboratory reaction tube and having a support element, on which oligonucleotide probes are immobilized on predetermined regions, arranged on one of its base areas for specifically detecting bacterial strains of the species *Pseudomonas aeruginosa*.

4. Device according to claim 2 or 3, characterized in that the oligonucleotide probes are selected in such a way that they detect 30% to 70% of the population of *Pseudomonas aeruginosa* strains in each case.

5. Device according to any one of claims 2 to 4, characterized in that the oligonucleotide probes are specific for nucleic acids having a base substitution compared to the sequence of the reference strain of *Pseudomonas aeruginosa*.

6. Device according to any one of claims 2 to 5, characterized in that the oligonucleotide probes are specific for nucleic acids present in only one or few strains of the species *Pseudomonas aeruginosa*.

7. Device according to any one of claims 2 to 6, characterized in that the oligonucleotide probes are specific for nucleic acids present in pathogenicity islets in the genome of *Pseudomonas aeruginosa*.

8. Device according to any one of claims 2 to 7, characterized in that the oligonucleotide probes are specific for nucleic acids present in disease-associated genes like *exoS* and *exoU*.

9. Device according to any one of claims 2 to 8, characterized in that the oligonucleotide probes are specific for nucleic acids contained in genes coding for flagella of *Pseudomonas aeruginosa*.

10. Device according to any one of claims 2 to 9, characterized in that the oligonucleotide probes are selected from the oligonucleotides according to claim 1.

11. Method for specifically detecting bacterial strains of the species *Pseudomonas aeruginosa* in a sample, comprising the following steps:

- a) contacting the sample with a nucleic acid chip in a microarray device according to any one of claims 2 to 10; and
- b) detecting the interaction between the oligonucleotide probes and the target nucleic acids contained in the sample.

12. Method according to claim 11, characterized in that the target nucleic acids contained in the sample are amplified before the detection.

13. Method according to claim 12, characterized in that the amplification is performed by means of multiplex PCR.

14. Method according to claim 13, characterized in that primers, which have similar melting points and / or similar binding kinetics, are used for the amplification.

15. Method according to any one of claims 12 to 14, characterized in that the amplification is performed linearly.

16. Method according to any one of claims 12 to 15, characterized in that the primers are selected with a nucleic acid sequence selected from the group consisting of (all sequences in 5' → 3' direction):

ACGCGGATGTCCTGGATTTGG

CTGAAGAAGGGGCGCTACGCGGCGTACCGGGCAAGGTGATAGCTCGGTGAAACA

TCGGGAGGGTCATCCAGCAAGCCATTGCGCGGAGTCGCTTTCCGCCATCGTGAG

TCGCTTTCCGCCATCGAAGGGCGTTTCACGCTGACGC

ATCCGGAAGGGCGTTTCACG
TCCACACCTCAGACTTCGGCG
TATTGACGACCTACCGCGCGC
GCAACTGATGTTGCGCCAGC
CGCAACTGATGTTGCGCCAGC
ACACGCAACTGATGTTGCGCC
TGTCCTCGGCTCAGTTCAACG
AACACCTTGGCGTTTGTCCC
GCAACACCTTGGCGTTTGTCC
TCAAGCTCGTTGTGGACCGC
GTTACGACGGCGTGCTGTCGG
ACGCAACGTATTCGGCGACCC
CGCAACGTATTCGGCGACCC
AGCTGATGGTATCGCCGTCGC
CTAGTGATCGCACCGGAGCC
AGCCTCGACACCGGTTCTCG
TCGTTTCATCCCCAGGCTTCG
ACCATCTCGTTTCATCCCCAGG
TTCTGAGCCCAGGACTGCTCG
TCGACGCGACGGTTCTGAGCC
TGACGTTCTCGCCGGTAGCG
CAGTAGCGGTACCGGTCTGCG
CAGTAGCGGTACCGGTCTGC
TTCCTCGCCGGCATAGTAGGC
CGAGGACGAGGCATCTTCCGG
GCAGGTAGCAGGTTTCCAGG
AACTGTTCTTCTGCGCGGCG
TGATCGGCTTGGTCTCGCAGG
GCTGATCGGCTTGGTCTCGC
GAGGCGTTCTGCTCGTGGTCG
TTTTTCCAGCATGCGCAGGG
GCTGGCTTTTTTCCAGCATGCG
TTGCGGCTGGCTTTTTTCCAGC

TTGGGATAGTTGCGGTTGGC
CGTAGGCGATCTTCACCCGC
TGGCGTAGGCGATCTTCACCC
GGCGAGATAGCCGAACAGGC
GCGGCGAGATAGCCGAACAGG
CACTTGCTGCTCCATGAGCC
GAGGTCGAGCAGGCTGATGC
TAGGTCGCGAGGTCGAGCAGG
GTCCTTCTGCACCGAGTCGG
CGCATCTTGTCTGGGTCAGG
TCGTCGAGGCGCATCTTGTCC
ACGTCGAGGTGGGTCTGTTCG
GTAGCCTTCGGCATCCAGCG
TCGGCATTGGGATAGTTGCGG
CCTCCTGTCTCATGCCGATGC
GCATTCGCCACGGAAGGAAGG
GAAGGCATCATGGCATTCGCC
GTCATGGGGTTTCCCAGAGACC
GATCGCGATGTCGACGGTGCC
CGATCGCGATGTCGACGGTGC
TGCCGATCGCGATGTCGACG
GACGAATACCCAGCTGCGTGG
GCAGACGAATACCCAGCTGCG
CGCGACGTCGTGACGTCAGC
ACTTTCGGCTCTTCGGGGCTGG
AGGTAGAGACTCGGGGGAACC
TCGTTTTTCGGTCATGGCCAGG
TTCCGCGACGAACATCCGTGG
CGCTTCCGCGACGAACATCCG
GGATCGCTTCCGATAGGGCAGC
AGAGGCATGGGTCTGTACCG
TCTGTCAATCCCCTTTGGGG
AGCCCCTTTCTGTCAATCCCC

GGCTTCCTACCGAAGGTCAGG
TGAGGGCTTCCTACCGAAGG
TTCAAGGTCATGGGCAATGCC
AGTCCCTTCAAGGTCATGGGC
GCCGACTGAGCTGTAGCTCGG
GGCCGACTGAGCTGTAGCTCG
ACCAGACTGGTCAATGGTGG
CCCGTGTTCCGTAGACCTTGC
AGCAGTTACCCACAGCATGG
CAGCAGTTACCCACAGCATGG
CTACACTCCAACCGCTGGTCC
GACCTACACTCCAACCGCTGG
TTCCCTTGCTGCCGAGAAGC
TAATAGGCGAGCCTGCCGTCC
TCCACGCCGAGGGACGTGCC
GCTCCACGCCGAGGGACGTGCC
CGCGGTGCTGGTTGCGCTGC
CCAATGCCCAGGGCCAGCGGA
CGCTGGCAGTTCCGCTGGCC
CAGGGTCGCCAGCTCGCTCGCC
AGGGTCGCCAGCTCGCTCGC
AGTGATCTGCCGCGGCCCTGCC
GTGATCTGCCGCGGCCCTGC
GTTCCACAGGCGCTGCGGCGC
GTTCCACAGGCGCTGCGGCG
CAAAGCCCCTGGTCGCGCGG
GCAGCTTTTCCACCGCCGGCGG
AAACTGCCCCGCCCCCATCC
GGAAAACTGCCCCGCCCCC
ACGCTCGCAGCGCCTCACGCG
GGCCTGGCTGCGAACGCTCGC
GGGGTCGAGACGTGTACATGG
TTCCTGGGCCAGAGTTGGACC

AGCTTAAGGCCGTGGCACTCG
CCGGAGAATTCGCGTCCACC
TGCTGACGATGAAGCCCCAGC
AGGAGGCCGATGACAACACCC
TGCCGATTCCATGCTCACGCC
ACGACGTCACCGTCGAGACCG
ACCGCCTTTCTGGTGAGCTGG
AGCCAAGACGGTTGTTCGCGG
TCAATGACGCCGAGTTGGCGC
CTCGGACAGGTTACGCTGG
GCCATTCGCTGCAACACCTCC
GCGCGCGTTCGAGAAACAGG
CGGAGGTTGAAAAGCTGGCCC
ATGCCATCGTTGAAGGCACCGC
TGCCATCGTTGAAGGCACCG
TCTGGCGGAATCAGGTAGGCC
CTTCCGGGGAGAAACCACCG
ACCTCCAGCACCGACACACC
ATCCGATCCACCTCCAGCACC
CGTTCAGGTCGTAGACCGCGC
GCGATACCAACTGTCCTGCGGC
TGCCGAAGGTGAATGGCTTGCC
CCTGATGGTCCGATCCCAGC
GCCGAGGGTCAAGAACCACTGG
TCTTGGCCCAGTCATAGCGGC
TAACCCCAAGGCCCATTTGGAGG
GCCACCGCCTTCGAATAACCCC
AATTGCTCGAGGGATGCGGC
GGTCGAAACGGATGCGCAGG
GCCCCGCGTCATTTTCACGTCG
AATGCTCTGGGCAACGAGCC
CTACCCAGCTTGGGCGTAGC
AAGCGATAGCCGTGCTCCTGC

CCGGCTATATCCGCGGCTACC
ATTGGCGCTGCTGTTTACGCCC
GGTGGCGTCGGGTTTTTCTGC
AGGTCGTAGCGGAAGGTGGTGG
ATCTGAACCGAGGGGATCCGC
CCCGGGAGTCATTGGTCTGG
GCCTGTTGGACCCCTTTGACC
TACTCCTGCCTGTTGGACCCC
CGCTCAAGCGCTATCCCACC
CGCCATCGGCCTGTACAACG
CGGTAGAGAGCTGGGTTGGC
AACCTGGAGCTAGGGCAGAGC
GGTGCTCGACCCAAGCATCG
TCCTTGAGTTCCTTGGCGCGG
CAACACGCGACTGGCGATCC
TACATCATCCGCAACGGCGGC
TATTGACGACCTACCGCGCGCC
CACCAAGAACCCGCTGCTCG
ATCGTGGCAGGATGTCCACCG
TAGGCGGGCCTTTTGAAGGTGC

17. Use of the oligonucleotides according to claim 1 for specifically detecting bacterial strains of the species *Pseudomonas aeruginosa*.

18. Use of the oligonucleotides according to claim 1 or of the device according to any one of claims 2 to 10 or of the method according to any one of claims 11 to 16 for genotyping and pathotyping *Pseudomonas aeruginosa*.

19. Use of the primers according to claim 16 for amplifying nucleic acids of bacterial strains of the species *Pseudomonas aeruginosa*.